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Investigations on the alleged goitrogenic properties of milk

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With 2 figures and 11 tables

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In 1956, CLEMENTS and WISHART (1) presented the hypothesis that the unexpected rise in goitre-prevalence in certain parts of Tasmania, five years after the introduction of iodine prophylaxes, might be caused by a goitrogenic factor transmitted to the human subject through milk from cows fed on marrowstem kale, "Choumoellier" (*Brassica oleracea* var. *moelleria*). Their paper also described

some experiments with rats and human subjects which seemed to support the hypothesis. Serious attention has been given in the literature to the idea that goitrogenic substances in milk could give cause to goitre. In the first book on endemic goitre published by the World Health Organization (WHO) in 1960, KELLY and SNEDDEN (2) in their extensive article on the prevalence and geographical distribution of endemic goitre report comprehensively the paper of CLEMENTS and WISHART, which contains also some experimental material to support the hypothesis. PELTOLA (3, 4) has advanced the opinion that in Finland endemic goitre is caused more by a goitrogenic factor than by iodine deficiency, a factor whose effect in analogy with the factor of CLEMENTS and WISHART is not inhibited by iodine and which is transferred to milk from cruciferous plants. His claim is based on feeding experiments with rats which have received milk from goitrous and non- goitrous areas.

Should milk, the inclusion of which is considered essential in the diet of adolescents, and which is therefore included in the school meals everywhere, really contain dangerous amounts of substances which inhibit the synthesis of thyroid gland hormones, it would be absolutely necessary to take measures to avert the danger. The evidence in favour of this concept is insufficient, however. That is why we began to investigate the problem more closely in 1958. Results of these investigations have been published earlier. One of us (AIV) (5) presented a summary of the results obtained in this laboratory in 1958–60. Because the last part of our investigations, an experiment with rats which lasted a year, was finished last autumn, the results of this experiment, as well as a short account of the results obtained previously in this field in our laboratory, are presented here. We consider that the problem about the alleged goitrogenic properties of milk has been elucidated to such an extent that our work in this field is finished at this stage at least.

L-5-Vinyl-2-thiooxazolidone as a possible antithyroid substance in milk

In 1949, ASTWOOD, GREER, and ETTLINGER (6) isolated L-5-vinyl-2-thiooxazolidone (VTO) from moistened and finely ground *Brassica* seeds. The antithyroid effect of this substance on man was as strong as that of propylthiouracil, which at that time was one of the most effective antithyroid substances. The substance was also isolated from crushed rutabaga. The effect of VTO (goitrin) was not suppressed by an adequate iodine intake, and hence it affected hormone synthesis. Other derivatives of thiooxazolidone were isolated from crushed and moistened seeds of cruciferous plants: dimethylthiooxazolidone from *Conringia orientalis* (7), and phenylthiooxazolidone from *Barbarea vulgaris* (8). After ETTLINGER and LUNDEEN (9) had demonstrated the mustard oil glucosides to be derivatives of hydroxylamine, and after the old structure formula proposed for this group of compounds by GADAMER (10) had been essentially revised, the formation of vinylthiooxazolidone (II) from the glucoside (progoitrin) (I) in which carbon atom 2 contains a hydroxyl group, can be considered to take place in the following way [GREER (11), KJAER et al (12)] (Fig. 1).

ASTWOOD et al. (6) were unable to demonstrate with the analytical methods they employed the formation of VTO in the crushed leaves of *Brassica* plants, and hence the occurrence of its precursor would have been restricted to the seeds or the underground parts of these plants. In this laboratory KREULA and KIESVAARA (13) developed a sensitive quantitative method for determination

of VTO. By this method the goitrogenic substance was found to be formed also in crushed cabbage and in the leaves and stems of numerous fodder plants belonging to the genus *Brassica* (14). The amounts of the precursors of VTO are much smaller in the green parts of the plants than in the seeds. It ought, however,

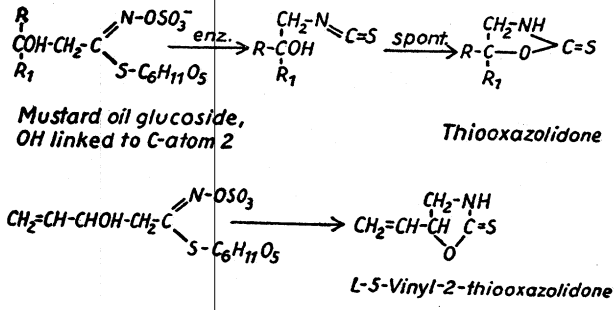

Figure 1. Vide text,

to be remembered that the dry matter content of the leaves is only about 10%. The VTO values found in crushed seeds and in the juice pressed from the leaf stems are shown in Tables 1 and 2 [KREULA and KIESVAARA (13)].

Table 1. VTO values found in crushed seeds of cruciferous plants

Species	Number of samples	VTO	
		Ranges of variation mg/g	Average mg/g
Spring rape	8	2.3 – 4.2	3.16
Winter rape	11	7.7 – 12.3	8.96
Spring turnip rape	3	0.2 – 0.8	0.46
Winter turnip rape	16	0.04 – 0.7	0.27
Big-leafed turnip	9	0.2 – 3.5	1.16
Turnip	2	0.5 – 0.9	0.70
Swede	9	3.2 – 14.5	6.70
Marrowstem kale	1	—	1.07
Radish	1	—	0.04

Table 2. VTO values of green parts of cruciferous plants. Samples from field cultivations and greenhouse. Contents given in µg/ml press juice

Species	Number of samples	VTO	
		Ranges of variation µg/ml	Average µg/ml
Spring rape V[1]	1	—	4
„ „ K[2]	8	2 – 40	14
Winter rape V	9	7 – 73	33
„ „ K	4	23 – 135	69
Spring turnip rape K	2	3 – 60	32
Winter „ „ K	1	—	24
Turnip K	1	—	72
Big-leafed turnip V	4	11 – 55	25
Marrowstem kale V	3	0 – 8	3
„ „ K	1	—	9

A cow eats about 30 kg of green rape and turnip rape daily and can thus receive 1 g of VTO in its daily ration. If a considerable fraction of this amount of VTO should be transferred from the cow's organism to the milk it could become goitrogenic. It should, however, be remembered that marrowstem kale, which was supposed by CLEMENTS and WISHART to make milk goitrogenic, contains very little of the precursor of VTO. CLEMENTS (15) also considered pasture weeds belonging to the family *Cruciferae* as potential goitrogens.

The problem of the transfer of VTO from fodder to milk now became important. It turned out that the determination of VTO in milk required special measures even because of the rapid disappearance of the substance from milk. The best method for the stabilization of VTO in milk is to heat it to 85 °C immediately after milking. The substance is then retained in the chilled milk for at least 24 h, and at —15 °C for several days. Later it was observed that the addition of hydrogen sulphide restores VTO, and hence its disappearance from unheated milk is probably due to a mild oxidation, caused by peroxidase, which can be eliminated by hydrogen sulphide. The results shown in Table 3 were obtained with milk which was heated to 85 °C immediately after milking (16).

Table 3. Transfer of VTO from rumen to milk. Experiments with cows

500 mg cryst. VTO							
Before VTO feeding 0	after	2 h		6.5 h	10.5 h	24 h	48 h
VTO content of milk µg/l		190		35	17	+ ?	0
VTO transferred to milk			0.035	0.013	0.006		
% of total fed				0.05			
100 g crushed, moistened seeds of spring rape (800 mg VTO)	after	12 h	24 h	36 h	48 h		
VTO content of milk µg/l		77	26	17	0		
% of total fed			0.02				
1.6 g cryst. progoitrin (525 mg VTO)	after	12 h					
VTO content of milk µg/l		traces					
2×15 kg marrowstem kale kg/day (66 mg VTO)	after	12 h					
VTO content of milk µg/l		4					
% of total fed		0.05					
10 kg green winter rape (280 mg VTO)	after	12 h					
VTO content of milk µg/l		20					
% of total fed		0.04					

The results show that about 0.05% of the VTO fed can be found in the milk. When crystalline VTO is fed, after 2 h about two-thirds of the total transferred VTO is found in the milk, and hence the substance apparently passes through the rumen wall into the blood. When the crystalline precursor-glucoside (progoitrin) of VTO was fed, at the most only traces of VTO were found in the milk, and hence either the glucoside is not hydrolyzed in the rumen without the myrosinase complex which occurs in plants or the VTO formed is subsequently decomposed in the rumen.

After these experiments it was obvious that such amounts of VTO are not transferred to milk from fodder plants that they could be of any importance as goitrogens in milk. When feeding cows with green cruciferous plants, the amount of VTO hardly reaches 100 μg of VTO per litre of milk. In fact, we never found such a high concentration as this in milk when green *Brassica* plants (green winter rape, marrowstem kale, and turnip rape) were fed to cattle *ad libitum*. The largest amount of VTO found in milk from different farms was 20 μg per litre. Since no effect on the iodine uptake of the thyroid gland can be demonstrated with 20 mg of VTO, this substance can be left out of consideration as a goitrogen in milk.

BACHELARD and TRIKOJUS (17) later isolated γ-methylsulphonylpropyl-*iso*-thiocyanate, cheirolin, from the fruit and leaves of turnip weed *(Rapistrum rugosum)*, and, by experiments on rats, proved it to be goitrogenic. Endemic goitre among children occurs only in the area in Southern Queensland where turnip weed grows profusely and this *iso*thiocyanate could therefore function as a goitrogen. GMELIN and VIRTANEN (5) fed 1 g of cheirolin to a milking cow but found no cheirolin or the thiourea formed from it, $(CH_3 \cdot SO \cdot CH_2 \cdot CH_2 \cdot NH)_2 C = S$, in the milk. This compound does not accordingly make milk goitrogenic either.

The thiocyanate ion (SCN^-) as a possible thyrostatic factor in milk

In 1928, CHESNEY et al. (18) observed that rabbits which received heavy cabbage feeding developed large goitres. In 1936, BARKER (19) observed that goitres developed in two patients who had been given potassium thiocyanate (KSCN) as a treatment for high blood pressure. ASTWOOD (20) found that the goitre caused by SCN^- is formed only if the iodine content of the food is low. Shortly afterwards it was proved that the SCN^- of the thyroid gland inhibits the uptake of iodine by this gland. When it was also found that "cabbage goitre" could be prevented by including enough iodide in the food, it seemed likely that this goitre was due to SCN^-. At first it was considered to be formed in the animal and human organism from organic cyanides or cyanogenic glucosides occurring in cabbage, until MICHAJLOVSKIJ and LANGER (21) in 1958 demonstrated that *Brassica* plants contained considerable amounts of preformed SCN^-.

In this laboratory no SCN^- was found in intact cabbage, but a precursor, from which SCN^- is rapidly formed, when cabbage was crushed or chewed [GMELIN and VIRTANEN (22)]. The precursor, glucobrassicin, was isolated in crystalline form as the tetramethylammonium salt [GMELIN, SAARIVIRTA, and VIRTANEN (23)]. The structure of the isolated compound was established and the formation of SCN^- explained [GMELIN and VIRTANEN (24, 25)]. Glucobrassicin was the first mustard oil glucoside found to contain the indole group, and that is why it has chemical and physiological properties which the numerous previously-known mustard oil glucosides lack. Its enzymatic and subsequent spontaneous cleavage reactions are shown in Fig. 2.

At a neutral reaction the splitting of SCN^- is quantitative, i.e. 1 mole of SCN^- is formed from 1 mole of glucobrassicin. The amount of glucobrassicin can thus be calculated on the basis of the quantitative estimation of SCN^- formed enzymatically in crushed plants. A derivative of glucobrassicin, N_1-methoxy-glucobrassicin, is, however, to be found in some *Brassica* plants [GMELIN and

VIRTANEN (26)]. This is split enzymatically, to give SCN^-, in analogy with glucobrassicin.

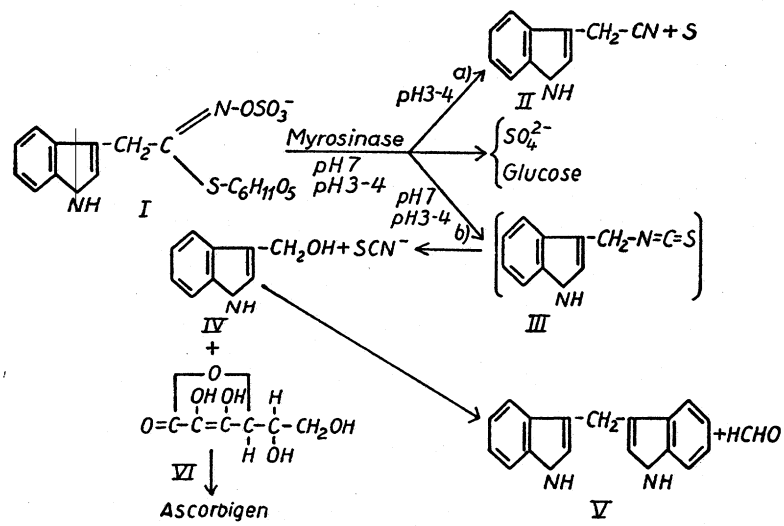

Figure 2. Vide text.

Normally, cow's milk contains 1 to 5 mg of SCN^- per litre. In our current feeding experiments with cows, (27, 28) in which purified starch, sucrose, and pure sulphite cellulose were used as the sources of energy, maize oil as the source of essential fatty acids, and urea plus a small amount of ammonium sulphate and phosphate as the sole sources of nitrogen, milk which contained no SCN^- at all was produced. The milk of cows fed on large amounts of *Brassica* plants usually contains 6 to 9 mg of SCN^- per litre. The content of SCN^- in milk depends, accordingly, on the presence of compounds (e.g. glucobrassicin) in the fodder from which SCN^- is split off by plant enzymes, or compounds (e.g. organic cyanides and cyanogen glucosides) which contain the CN group which is detoxicated to SCN^- in the cow's liver.

GMELIN and VIRTANEN (29) fed large doses of potassium and ammonium thiocyanate and estimated SCN^- in milk in feeding experiments with cows. When as much as 6 g of SCN^- was fed daily during 4 days to a cow, the milk contained 14 to 12 mg of SCN^- per litre. A daily dose of 3 g of SCN^-, which the cow can obtain when large amounts of *Brassica* plants are fed, increased the SCN^- content of milk to 8 or 10 mg per litre. This is in practice the highest SCN^- content which cow's milk can have. Estimations in this laboratory on the effect of SCN^- on thyroidal ^{131}I uptake in man showed that 181 mg of SCN^- was the lowest amount which weakly retarded the uptake of iodide. 205 mg of SCN^- had no effect on another test subject. The effect is strong only from 300 mg upwards (30). The small amount of SCN^- occurring in milk cannot thus interfere with the iodine uptake of the thyroid gland in man, except perhaps in case the iodine content of the food is on a deficiency level. It is then, however, a question of iodine deficiency in the food. The effect of SCN^- in decreasing the iodine content of milk is noteworthy (31).

The dependence of the goitrogenic effect of SCN^- on the iodine content of the food is shown in Table 4 where the results of our experiments with rats which lasted 84 days are illustrated. The inhibiting effect of SCN^- on the uptake of iodine, on the amount of iodine bound in the serum protein, and on the weight of the thyroid glands, is clearly seen in the group which received a small amount of iodine (2 γ I/rat/day).

Table 4. Dependence of the goitrogenic effect of SCN^- on the iodine content of the food. Experiment with rats August 23 – November 14, 1960, 6 groups à 10 animals. Basal food the same in all groups

	Group A	2 µg iodine/rat/day		Group B	16 µg iodine/rat/day	
	Control	0.64 mg SCN^-/day	5.8 mg SCN^-/day	Control	0.70 mg SCN^-/day	7.5 mg SCN^-/day
Weight of rats, g	136.3	133.6	129.1	129.7	134.5	135.0
Weight of thyroid gland	15.8	19.7	27.1	8.6	9.8	10.7
mg/100 g rat	11.6	14.7	21.0	6.6	7.3	7.9
I in thyroid glands and in blood serum						
µg I/mg thyroid gland	0.060	0.041	0.022	1.16	1.06	0.58
µg I/100 ml serum	1.7	1.1	1.2	6.8	6.0	7.5
µg PBI/100 ml serum	1.7	1.2	1.2	3.2	3.5	3.8
Microscopic analysis of thyroid preparations						
Colloid	5.9%	5.8%	0.9%	30.8%	22.7%	22.8%
Epithel	79.4%	85.4%	88.8%	47.8%	59.8%	61.4%
Stroma	14.7%	8.8%	10.3%	21.4%	17.5%	15.8%

Unknown substances as possible antithyroid factors in milk

The transfer of some known goitrogenic factor to milk in such amounts that it could interfere with the function of the thyroid gland, when plenty of milk is used, could accordingly not be demonstrated in our experiments. There was still the possibility that some unknown factor could make milk goitrogenic.

In experiments with rats which lasted some months, and in which different groups of rats were fed on milk produced on different feeds, not the slightest indication of the transfer of such a factor to milk was obtained. CLEMENTS and WISHART (1) based their conception of the goitrogenic properties of milk not only on the increase of goitre-prevalence among school children in certain regions in spite of iodine therapy – which, of course, may be due to quite other causes than the supposed goitrogenic factors in milk – but also on some experiments with human subjects and rats in which the effect of milk on the iodine uptake of the thyroid gland was determined using ^{131}I as an indicator. In their experiments with rats, the authors injected subcutaneously an evaporated ethanol extract of milk and found this to inhibit the uptake of iodine strongly. We performed corresponding experiments, following the extraction method of these authors as much as possible, and found that the effect of the ethanol extract is due to salts + lactose which pass into it from the milk. By dissolving pure salts + lactose in water to about the same concentrations found by analysis in the ethanol extract of milk, a solution was obtained which, when injected into the rats, also inhibited the uptake of iodine strongly. The high

SCN^- content of the extract prepared from milk produced on a feed containing large amounts of marrowstem kale was the reason why this extract inhibited the uptake of iodine much more strongly than the corresponding extract of milk produced on other feed. This is shown in Table 5.

Table 5. The influence of subcutaneous injection of ethanol extracts of milk and a synthetic salt mixture on the uptake of ^{131}I compared with the effect of the injection of water. The volume of the solution injected was 1 ml/rat. The inhibition of the iodine uptake is expressed as per cent of the control (water injection). The number of rats in each group was 2×5.

	Extracts corresponding to mls milk unpasteurized			
	62.5	125	250	500
Extract of milk[1] (SCN^- 188 µg/250 ml milk)	9	31	52	75
Extract of milk rich in SCN^- (SCN^- 1600 µg/250 ml milk)	47	53	88	—
Synthetic salt mixture[2] corresp. to ethanol extract of 250 ml milk (without SCN^-)			29	
The same mixture + 10 µg I^-			40	
Synthetic salt mixture corresp. to ethanol extract of 500 ml milk (without SCN^-)				59

[1]) The composition of the ethanol extracts is given in the experimental part.
[2]) The salt mixture contained lactose, Na, K, Ca, and Cl (cf. experimental part).

The influence of SCN^- on the uptake of ^{131}I is shown in Table 6, which presents results of an experiment in which 833 ml milk poor in SCN^- and 133 ml milk rich in SCN^- were extracted with ethanol. The former extract contained 980 µg and the latter one 1080 µg SCN^-. An aqueous solution of 1000 µg SCN^- was used as control. Thus it was possible to estimate the effect of SCN^- and other salts on the uptake of radio iodine by the thyroid gland.

Table 6. The influence of subcutaneous injection of ethanol extracts of unpasteurized milk (833 ml milk extracted) and milk from a cow fed 6 g SCN^-/day (133 ml milk extracted) on the uptake of ^{131}I compared with the effect of the injection of water. The volume of the solution injected was 1 ml/rat. The inhibition of the iodine uptake is expressed as per cent of the control (water injected). The number of rats in each group was 2×5. The iodine fractions were separated on a paper chromatogram

Iodine fraction	SCN^- 1000 µg in aqueous solution	Extract of 833 ml milk containing 980 µg SCN^-	Extract of 133 ml milk rich in SCN^- containing 1080 µg SCN^-
1. Iodide	60	89	80
2. Thyroxine	50	97	76
3. Monoiodothyrosine	56	91	75
4. Di-iodothyrosine	62	95	79
Total	60	93	78

The extract prepared from 833 ml milk poor in SCN^- contained about 10 per cent less SCN^- than the extract of 133 ml milk rich in SCN^- but the salt + lactose concentration of the former was so much higher that the inhibition of radio iodine uptake caused by it exceeded the effect of the latter.

In some experiments with human subjects in which they, one hour after having taken a dose of ^{131}I, drank as much milk as they could, CLEMENTS and WISHART observed that the uptake of radio iodine was inhibited if the milk was produced on a liberal feeding of marrowstem kale. Milk which was not produced on cruciferous plants had no such effect. Unfortunately the experiments with different milk samples were performed only with one or two persons. In experiments performed in our laboratory with 21 persons [VILKKI, KREULA, and PIIRONEN (30)], and in which the milk samples used were produced on many different kinds of feeding (e.g. six different *Brassica* plants), the uptake of radio iodine by the thyroid gland was never observed to be inhibited by the effect of milk. The method used for the determination of ^{131}I was sensitive and reliable, a fact which was checked by letting the test person take small amounts of known antithyroid substances after no effect of the milk could be shown. An inhibition in the uptake of radio iodine then occurred regularly. The results of CLEMENTS and WISHART were accordingly not corroborated.

In the experiments performed in our laboratory, it turned out that the method of determining the radio-iodine uptake by the human thyroid gland was very sensitive to fluctuations in iodine supply. Some years ago, STANBURY et al. (32) reported that an increase of the carrier iodide dose in the uptake test exceeding 1.5 mg resulted in an abrupt fall in the ^{131}I uptake. If one now works in a range where the thyroidal capacity for iodide is already almost completely satisfied because of a plentiful supply of iodine, even very small additions of iodide may influence the form of the ^{131}I accumulation curve. Such a situation is especially likely to occur in communities where large doses (10 mg) of iodide are supplied at infrequent intervals for the prophylaxis of goitre. This was the case in the schools in Tasmania [CLEMENTS and WISHART (1)]. Failure to check the iodine balance might be the explanation of their reports on the goitrogenic effect of milk.

Next, we investigated PELTOLA's (3, 4) claim that the milk collected in a goitrous area contains a goitrogen the effect of which could not be eliminated by iodine ingestion in excess. As early as the 1930's, VIRTANEN and VIRTANEN (33) came to the conclusion, on the basis of iodine estimations in urine from goitrous and non-goitrous areas, that endemic goitre in Finland is caused by iodine deficiency. The estimations of VILKKI (34) in the 1950's on the iodine content of different foodstuffs in Southern and Central Finland led to the same conclusion. On the basis of his investigations, LAMBERG (35) considers that endemic goitre in Finland is due to the too low iodine content of the food.

PELTOLA's claim is primarily based on three feeding experiments with rats. The test animals were fed over a long period (1 to 2 years) in parallel groups on the same basal diet, one group receiving in addition milk from a goitrous district and another from a non-goitrous district. The weight of the thyroid glands of the animals was determined. In the last experiment also the ^{131}I uptake of the thyroid gland was determined.

In the first experiment, which lasted from March 1955 to February 1957, the rats (21 in number) received 14.3 and 14.2 μg of iodine daily. The milk for 11 animals was brought daily from a dairy located "in a moderately severe goitre endemia area" (Orimattila). The remaining 10 rats received milk from a dairy in a small town (Porvoo) located in a non-goitrous district. The distance between the dairies is 45 km. Each animal ingested on the average about 20 g of

milk per day. The weight of the animals at the end of the long experimental period was in both groups similar (336 ± 10 g and 325 ± 8.4 g), but the average weight of the thyroid gland was 41.3 ± 2.4 mg in the group (11 animals) which received milk from the goitrous district, and, from the non-goitrous district (10 animals), 25.9 ± 1.1 mg.

PELTOLA's second experiment (February 1958 to March 1959) was similar to the first. The only difference was that the total intake of iodine was now extremely large, 150.3 and 150.2 μg per animal per day. The weight of the animals (8 rats in both groups) at the end of the experimental period was 363 ± 4.8 g and 354 ± 13.5 g and of the thyroid glands 45.3 ± 2.0 mg (milk from the goitrous district) and 27.5 ± 1.0 mg (milk from the non-goitrous district).

In a third experiment, PELTOLA and KRUSIUS repeated the latter test. The number of animals was 46, but in both groups of test animals 4 to 6 rats were killed after 1, 3, 5, and 11 weeks, and then after a year. Already after one week the difference in the weight of the thyroid glands in both groups was highly significant (13.1 ± 0.5 mg and 9.6 ± 0.7 mg), – a very unexpected finding. After one year, when the groups comprised only 4 and 5 rats, the average weight of the thyroid glands was 43.8 ± 4.0 mg (milk from the goitrous district) and 26.8 ± 1.5 mg (milk from the non-goitrous district). The ^{131}I uptake of the thyroid glands per unit weight was at the end of the experiment about the same in both groups.

PELTOLA thus regularly found that milk from a moderately severe goitre endemic area increases the weight of the thyroid glands of rats during an experimental time of one or two years by 60 to 65%. This enlargement could not be prevented by iodine. The results were alarming, and at the same time they were inconsistent with our results concerning the transfer of different types of goitrogenic substances from plants to milk. We were therefore obliged to arrange a feeding experiment with rats lasting one year and in which one group of animals received milk brought daily from the same dairy (Orimattila) in a goitrous area as in PELTOLA's experiments. 80 female rats in all, and 5 groups were used in the experiment. The results are shown in Tables 7 and 8.

Table 7. Feeding experiment with 5 groups of rats, Oct. 23, 1961–Oct. 9, 1962. Additions to the basal diet in different groups were: dairy milk (Orimattila); milk of two cows fed with 30 kg marrowstem kale; casein in place of milk; water only

Additions to basic food	Exp. time days	milk fed ml/rat/day	water ml/rat/day	tot. protein g/rat/day	food g/rat/day	SCN^- μg/rat/day	Iodine μg/rat/day
dairy milk	350	21.7	0.4	2.15	9.43[2])	45	15.7
cow Eri „	350	21.9	0.1	2.17	8.92[2])	174	14.4
cow Lella „	350	21.7	0.1	2.26	9.53[2])	131	15.9
casein[1]	351	0	18.0	4.69	12.48	0	14.1
water	351	0	15.3	1.81	11.66	0	17.8

[1]) 3.26 g/rat/day, [2]) milk not included

Analyses showed that the milk of cows fed with marrowstem kale contained 3 to 4 times more SCN^- than the milk from the Orimattila dairy.

As is seen from Table 7 and Table 8, the average weight of the thyroid glands in the three groups which received milk was 16.1 to 18.6 mg (7.0 to 8.0 mg/100 g body wt). In the casein group the corresponding weight was 17.6 mg (7.0 mg/

Table 8. Results of the feeding experiment with 5 groups of rats (cf. Table 7)

	Milk of			Group with casein	Group with water
	Orimattilla dairy	Cow Eri[1])	Cow Lella[2])		
Number of animals	20	8	9	19	19
Weight of the animals, g/rat	229.3±7.3	229.3±1.2	232.0±6.9	252.9±7.3	224.3±6.1
Weight of the thyroid glands, mg/rat	17.6±0.92	16.1±0.96	18.6±1.3	17.6±0.92	20.0±0.76
Weight of the thyroid glands, mg/100 g body wt	7.7	7.0	8.0	7.0	8.9
Microscop. analyses of thyroid gland stroma	29.0±1.4	26.4±2.6	24.4±1.1	27.1±0.8	26.6±0.9
epithel	40.0±1.3	35.0±2.4	34.6±2.2	39.1±1.2	37.4±1.7
colloid	31.0±1.8	38.6±3.7	41.0±2.2	33.8±1.7	36.0±1.6
Iodine content of thyroid gland					
µg I/segment	11.2	10.0	12.5	12.2	13.7
µg I/gland	21.9	19.4	26.4	23.1	26.2
µg I/mg gland	1.25	1.21	1.42	1.31	1.31
Uptake of ^{131}I of thyroid gland					
c/min/gland	11.632±850	14.670±2200	19.600±3200	10.395±840	15.228±1900
% of given ^{131}I	4.7±0.34	5.9±0.88	7.5±1.28	4.2±0.34	6.1±0.76
c/min/mg gland	661±48	911±130	1054±170	590±48	761±95
Iodine in serum					
µg tot. I/100 ml	23.5	20.0	18.6	17.9	20.9
µg SBI/100 ml	2.9	2.7	2.5	2.7	3.7

[1]) Milk produced on a feed including 30 kg of marrowstem kale. Milk preserved frozen in small bottles at —20 °C. The fresh marrowstem kale fed to cow Eri was Chou-moellier (seed imported from Australia). This milk was fed to the group of rats during 251 days, when it became exhausted. After that milk produced on a feed including 30 kg of fresh marrowstem kale (seed imported from England) was fed to the group.

[2]) Milk produced with fresh marrowstem kale (seed imported from England) was fed to this group of rats throughout the experiment.

100 mg body wt) and in the water group 20.0 mg (8.9 mg/100 g body wt). Thus none of the types of milk fed caused an enlargement of the thyroid gland. The histological structure of the thyroid gland was similar in all groups. The uptake of ^{131}I injected 100 μc intraperitonially 24 h before killing the animals was not increased in the group with Orimattila milk. The groups with Orimattila and with casein show the lowest uptake, the groups with milk from cows fed on marrowstem kale the highest. Individual variations in these groups are, however, so great that the differences in the ^{131}I uptake between different groups are not significant. There is, however, a tendency of the groups with marrowstem kale milk towards an excited uptake of radioactive I^-. This is well understood because the feeding at the end of the experiment was terminated 24 h before the killing of the animals. As a consequence of the ending of the intake of SCN^- the competition between SCN^- and I^- was lessened and the uptake of ^{131}I could be increased.

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1. Skimmed milk was concentrated in a vacuum and then lyophilized. The powder obtained was extracted with abs. ethanol in portions of 60 g in a Soxlet apparatus under reduced pressure. After evaporation in a vacuum the residue was dissolved in a small amount of water.

Table 9. Composition of the ethanol extracts prepared from 833 ml dairy milk poor in SCN^- and from 133 ml experimental milk rich in SCN^-

	milk of cow Mila	milk from Valio's dairy
SCN^-	1080 μg[2])	980 μg[3])
dry subst.	0.20 g	1.33 g
salt equiv. as NaCl[1]	65.8 mg	564.0 mg
lactose	36.0 „	260.0 „
ash	66.4 „	506.0 „
P_2O_5	1.2 „	11.0 „
Na^+	20.0 „	102.0 „
K^+	11.0 „	102.0 „
Ca^{2+}	2.4 „	16.8 „
Cl^-	42.1 „	308.0 „
I^-	6.5 μg	17.0 μg

[1]) Found by conductivity measurements
[2]) 62% of the amount in milk
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2. Proteins were precipitated by the addition of ethanol to the milk. The filtrate obtained was concentrated in a vacuum. The main part of salts and lactose was precipitated by the addition of abs. ethanol. The fat was extracted with petrol ether and the solution concentrated in a vacuum until 1 ml corresponded to 250 or 500 ml of milk. 1 ml of the solution was always used for the injection into a rat. By diluting the solution, extracts could be prepared which corresponded to smaller amounts of milk (e.g. 62.5, 125, 250 ml milk).

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The influence of these extracts on the radio-iodine uptake when injected subcutaneously into rats is shown in Table 6. The ^{131}I dose was 100 μc and the time of action 4 h. The iodine fractions were separated on a one-dimensional paper chromatogram using collidine-water (NH_3) and butanol-ethanol-NH_4OH as solvents.

Using extraction method 2, extracts were prepared of 1 ml each from 500 ml of milk, one poor in SCN^-, the other rich in SCN^-. The composition of the extracts was as follows:

Table 10. Composition of two ethanol extracts of 1 ml each prepared from 500 ml milk

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The influence of these extracts in different dilutions on the radio-iodine uptake when injected subcutaneously into rats is shown in Table 5. The ^{131}I dose was 100μc, and the time of action 4 h.

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In order to investigate the effect of the ethanol extracts of milk on the uptake of ^{131}I by the thyroid gland, an aqueous solution of the salts and lactose found analytically in these extracts was prepared. The "synthetic" solution corresponding to the ethanol extract of 500 ml of milk had the following composition:

total solids	353.0 mg/ml
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K^+	30.0 „
Ca^{2+}	0.6 „
Cl^-	119.0 „
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The solution corresponding to the ethanol extract of 250 ml of milk was prepared by dilution with water.

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The test animals were female white rats (Spraque Dawley), the weight of which v about 60 g at the beginning of the experiments. The animals were placed in pairs into cag

Light and temperature conditions were much the same throughout the experime A constant temperature of 20 °C was maintained by thermostatic control. At two wee intervals the cages with test animals were moved in order that possible small differen in the test conditions, light especially, should be eliminated.

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In addition to dry food, the animals received tap water *ad libitum*. The milk grou received their daily portion of milk. In addition to the basal ration, the casein group rece ed 900 g of acid casein, mixed in 2500 g of dry food. The daily consumption of food was m sured by weighing the food rations and the remainders. The liquid consumption was a measured.

The composition of the food mixture was as follows:

rye flour	300 g
rolled oats	400 „
wheat embryos	200 „
casein	20 „
margarine (rendered fat)	50 „
cod liver oil	2 „
NaCl	10 „
Ca-lactate	10 „
Fe^{+++}-citrate	300 mg
$MgSO_4$	100 „
$CuSO_4$	20 „
KI	2 „
Total	992. 422 g

The food mixture contained 2.48% of nitrogen corresponding to 15.5% of raw prote The iodine content of the diet without KI and addition of milk was 0.5 μg per 10 g feed

The test animals received an additional ration of yeast once a week, primarily to supp ment the vitamin requirement.

Methods of analysis

The contents of the stroma, epithel, and colloid of the thyroid glands were determir by microscopy of thyroid gland sections prepared according to the method of ERÄNKÖ (3 One of the thyroid gland segments of each rat was used for this purpose.

The total iodine in the thyroid glands was analyzed in the other segment of the thyr gland from a hydrolyzate prepared with polidase-S. The iodine analysis was performed usi a modification of the method of BARKER et al. (37) introduced by PIIRONEN and VIRTAN (31).

The total iodine and PBI of the serum were measured according to the method VILKKI, KREULA, and PIIRONEN (30).

In the ^{131}I experiments 100 μc of ^{131}I (carrier free) was injected intraperitonially ir the rat in 1 ml of water. After this the animals received no food before they were killed af 24 h, but they were allowed to drink water *ad libitum*.

The ^{131}I activity of the thyroid glands was measured in the excised thyroid gland s ments with a Baird Atomic γ-spectrometer. A big (surface) crystal was used, upon whi the glands were placed in a glass jar with a lid. Each test animal was analyzed separate The aliquot of the solution injected was measured using the same equipment.

The total ^{131}I activity of the serum and the ^{131}I in the PBI fraction were measured by the γ-spectrometer using a cavity crystal.

The SCN^- content of the milk was determined according to the method of PIIRONEN and VIRTANEN (31).

The SCN^- and I contents of the test milk samples are shown in Table 11.

Table 11. SCN^-- and I-content of milk samples

Milk	SCN–		I	
	range mg/l	average mg/l	range μ/l	average μg/l
Orimattila dairy	1.2–5.0	2.1	30– 95	63.6
Cow Eri	7.6–9.2	7.9	29– 57	40.4
Cow Lella	5.5–6.8	6.0	28–106	62.7

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In some experiments with human subjects in which they, one hour after having taken a dose of ^{131}I, drank as much milk as they could, CLEMENTS and WISHART observed that the uptake of radio iodine was inhibited if the milk was produced on a liberal feeding of marrowstem kale. Milk which was not produced on cruciferous plants had no such effect. Unfortunately the experiments with different milk samples were performed only with one or two persons. In experiments performed in our laboratory with 21 persons [VILKKI, KREULA, and PIIRONEN (30)], and in which the milk samples used were produced on many different kinds of feeding (e.g. six different *Brassica* plants), the uptake of radio iodine by the thyroid gland was never observed to be inhibited by the effect of milk. The method used for the determination of ^{131}I was sensitive and reliable, a fact which was checked by letting the test person take small amounts of known antithyroid substances after no effect of the milk could be shown. An inhibition in the uptake of radio iodine then occurred regularly. The results of CLEMENTS and WISHART were accordingly not corroborated.

In the experiments performed in our laboratory, it turned out that the method of determining the radio-iodine uptake by the human thyroid gland was very sensitive to fluctuations in iodine supply. Some years ago, STANBURY et al. (32) reported that an increase of the carrier iodide dose in the uptake test exceeding 1.5 mg resulted in an abrupt fall in the ^{131}I uptake. If one now works in a range where the thyroidal capacity for iodide is already almost completely satisfied because of a plentiful supply of iodine, even very small additions of iodide may influence the form of the ^{131}I accumulation curve. Such a situation is especially likely to occur in communities where large doses (10 mg) of iodide are supplied at infrequent intervals for the prophylaxis of goitre. This was the case in the schools in Tasmania [CLEMENTS and WISHART (1)]. Failure to check the iodine balance might be the explanation of their reports on the goitrogenic effect of milk.

Next, we investigated PELTOLA's (3, 4) claim that the milk collected in a goitrous area contains a goitrogen the effect of which could not be eliminated by iodine ingestion in excess. As early as the 1930's, VIRTANEN and VIRTANEN (33) came to the conclusion, on the basis of iodine estimations in urine from goitrous and non-goitrous areas, that endemic goitre in Finland is caused by iodine deficiency. The estimations of VILKKI (34) in the 1950's on the iodine content of different foodstuffs in Southern and Central Finland led to the same conclusion. On the basis of his investigations, LAMBERG (35) considers that endemic goitre in Finland is due to the too low iodine content of the food.

PELTOLA's claim is primarily based on three feeding experiments with rats. The test animals were fed over a long period (1 to 2 years) in parallel groups on the same basal diet, one group receiving in addition milk from a goitrous district and another from a non-goitrous district. The weight of the thyroid glands of the animals was determined. In the last experiment also the ^{131}I uptake of the thyroid gland was determined.

In the first experiment, which lasted from March 1955 to February 1957, the rats (21 in number) received 14.3 and 14.2 μg of iodine daily. The milk for 11 animals was brought daily from a dairy located "in a moderately severe goitre endemia area" (Orimattila). The remaining 10 rats received milk from a dairy in a small town (Porvoo) located in a non-goitrous district. The distance between the dairies is 45 km. Each animal ingested on the average about 20 g of

milk per day. The weight of the animals at the end of the long experimental period was in both groups similar (336 ± 10 g and 325 ± 8.4 g), but the average weight of the thyroid gland was 41.3 ± 2.4 mg in the group (11 animals) which received milk from the goitrous district, and, from the non-goitrous district (10 animals), 25.9 ± 1.1 mg.

PELTOLA's second experiment (February 1958 to March 1959) was similar to the first. The only difference was that the total intake of iodine was now extremely large, 150.3 and 150.2 μg per animal per day. The weight of the animals (8 rats in both groups) at the end of the experimental period was 363 ± 4.8 g and 354 ± 13.5 g and of the thyroid glands 45.3 ± 2.0 mg (milk from the goitrous district) and 27.5 ± 1.0 mg (milk from the non-goitrous district).

In a third experiment, PELTOLA and KRUSIUS repeated the latter test. The number of animals was 46, but in both groups of test animals 4 to 6 rats were killed after 1, 3, 5, and 11 weeks, and then after a year. Already after one week the difference in the weight of the thyroid glands in both groups was highly significant (13.1 ± 0.5 mg and 9.6 ± 0.7 mg), – a very unexpected finding. After one year, when the groups comprised only 4 and 5 rats, the average weight of the thyroid glands was 43.8 ± 4.0 mg (milk from the goitrous district) and 26.8 ± 1.5 mg (milk from the non-goitrous district). The ^{131}I uptake of the thyroid glands per unit weight was at the end of the experiment about the same in both groups.

PELTOLA thus regularly found that milk from a moderately severe goitre endemic area increases the weight of the thyroid glands of rats during an experimental time of one or two years by 60 to 65%. This enlargement could not be prevented by iodine. The results were alarming, and at the same time they were inconsistent with our results concerning the transfer of different types of goitrogenic substances from plants to milk. We were therefore obliged to arrange a feeding experiment with rats lasting one year and in which one group of animals received milk brought daily from the same dairy (Orimattila) in a goitrous area as in PELTOLA's experiments. 80 female rats in all, and 5 groups were used in the experiment. The results are shown in Tables 7 and 8.

Table 7. Feeding experiment with 5 groups of rats, Oct. 23, 1961–Oct. 9, 1962. Additions to the basal diet in different groups were: dairy milk (Orimattila); milk of two cows fed with 30 kg marrowstem kale; casein in place of milk; water only

Additions to basic food	Exp. time days	milk fed ml/rat/day	water ml/rat/day	tot. protein g/rat/day	food g/rat/day	SCN^- μg/rat/day	Iodine μg/rat/day
dairy milk	350	21.7	0.4	2.15	9.43[2])	45	15.7
cow Eri ,,	350	21.9	0.1	2.17	8.92[2])	174	14.4
cow Lella ,,	350	21.7	0.1	2.26	9.53[2])	131	15.9
casein[1]	351	0	18.0	4.69	12.48	0	14.1
water	351	0	15.3	1.81	11.66	0	17.8

[1]) 3.26 g/rat/day, [2]) milk not included

Analyses showed that the milk of cows fed with marrowstem kale contained 3 to 4 times more SCN^- than the milk from the Orimattila dairy.

As is seen from Table 7 and Table 8, the average weight of the thyroid glands in the three groups which received milk was 16.1 to 18.6 mg (7.0 to 8.0 mg/100 g body wt). In the casein group the corresponding weight was 17.6 mg (7.0 mg/

Table 8. Results of the feeding experiment with 5 groups of rats (cf. Table 7)

	Milk of			Group with casein	Group with water
	Orimattilla dairy	Cow Eri[1])	Cow Lella[2])		
Number of animals	20	8	9	19	19
Weight of the animals, g/rat	229.3±7.3	229.3±1.2	232.0±6.9	252.9±7.3	224.3±6.1
Weight of the thyroid glands, mg/rat	17.6±0.92	16.1±0.96	18.6±1.3	17.6±0.92	20.0±0.76
Weight of the thyroid glands, mg/100 g body wt	7.7	7.0	8.0	7.0	8.9
Microscop. analyses of thyroid gland stroma	29.0±1.4	26.4±2.6	24.4±1.1	27.1±0.8	26.6±0.9
epithel	40.0±1.3	35.0±2.4	34.6±2.2	39.1±1.2	37.4±1.7
colloid	31.0±1.8	38.6±3.7	41.0±2.2	33.8±1.7	36.0±1.6
Iodine content of thyroid gland					
µg I/segment	11.2	10.0	12.5	12.2	13.7
µg I/gland	21.9	19.4	26.4	23.1	26.2
µg I/mg gland	1.25	1.21	1.42	1.31	1.31
Uptake of ^{131}I of thyroid gland					
c/min/gland	11.632±850	14.670±2200	19.600±3200	10.395±840	15.228±1900
% of given ^{131}I	4.7±0.34	5.9±0.88	7.5±1.28	4.2±0.34	6.1±0.76
c/min/mg gland	661±48	911±130	1054±170	590±48	761±95
Iodine in serum					
µg tot. I/100 ml	23.5	20.0	18.6	17.9	20.9
µg SBI/100 ml	2.9	2.7	2.5	2.7	3.7

[1]) Milk produced on a feed including 30 kg of marrowstem kale. Milk preserved frozen in small bottles at —20 °C. The fresh marrowstem kale fed to cow Eri was Chou-moellier (seed imported from Australia). This milk was fed to the group of rats during 251 days, when it became exhausted. After that milk produced on a feed including 30 kg of fresh marrowstem kale (seed imported from England) was fed to the group.

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Feeding

In addition to dry food, the animals received tap water *ad libitum*. The milk grou received their daily portion of milk. In addition to the basal ration, the casein group rece ed 900 g of acid casein, mixed in 2500 g of dry food. The daily consumption of food was m sured by weighing the food rations and the remainders. The liquid consumption was a measured.

The composition of the food mixture was as follows:

rye flour	300 g
rolled oats	400 „
wheat embryos	200 „
casein	20 „
margarine (rendered fat)	50 „
cod liver oil	2 „
NaCl	10 „
Ca-lactate	10 „
Fe^{+++}-citrate	300 mg
$MgSO_4$	100 „
$CuSO_4$	20 „
KI	2 „
Total	992. 422 g

The food mixture contained 2.48% of nitrogen corresponding to 15.5% of raw prote The iodine content of the diet without KI and addition of milk was 0.5 μg per 10 g feed

The test animals received an additional ration of yeast once a week, primarily to supp ment the vitamin requirement.

Methods of analysis

The contents of the stroma, epithel, and colloid of the thyroid glands were determir by microscopy of thyroid gland sections prepared according to the method of ERÄNKÖ (3 One of the thyroid gland segments of each rat was used for this purpose.

The total iodine in the thyroid glands was analyzed in the other segment of the thyr gland from a hydrolyzate prepared with polidase-S. The iodine analysis was performed usi a modification of the method of BARKER et al. (37) introduced by PIIRONEN and VIRTAN (31).

The total iodine and PBI of the serum were measured according to the method VILKKI, KREULA, and PIIRONEN (30).

In the ^{131}I experiments 100 μc of ^{131}I (carrier free) was injected intraperitonially ir the rat in 1 ml of water. After this the animals received no food before they were killed af 24 h, but they were allowed to drink water *ad libitum*.

The ^{131}I activity of the thyroid glands was measured in the excised thyroid gland s ments with a Baird Atomic γ-spectrometer. A big (surface) crystal was used, upon wh the glands were placed in a glass jar with a lid. Each test animal was analyzed separate The aliquot of the solution injected was measured using the same equipment.

The total ^{131}I activity of the serum and the ^{131}I in the PBI fraction were measured by the γ-spectrometer using a cavity crystal.

The SCN^- content of the milk was determined according to the method of PIIRONEN and VIRTANEN (31).

The SCN^- and I contents of the test milk samples are shown in Table 11.

Table 11. SCN^-- and I-content of milk samples

Milk	SCN–		I	
	range mg/l	average mg/l	range μ/l	average μg/l
Orimattila dairy	1.2–5.0	2.1	30– 95	63.6
Cow Eri	7.6–9.2	7.9	29– 57	40.4
Cow Lella	5.5–6.8	6.0	28–106	62.7

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